PharmaNutrition 10 (2019) 100163

Contents lists available at ScienceDirect

PharmaNutrition

journal homepage: www.elsevier.com/locate/phanu

# Non-digestible oligosaccharides partially prevent the development of LPS-induced lung emphysema in mice

Hamed Janbazacyabar[a], Jeroen van Bergenhenegouwen[a,b], Kim A.T. Verheijden[a], Thea Leusink-Muis[a], Ardy van Helvoort[b,c], Johan Garssen[a,b], Gert Folkerts[a], Saskia Braber[a,*]

[a] Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, the Netherlands
[b] Danone Nutricia Research, Utrecht, the Netherlands
[c] Department of Respiratory Medicine, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Centre, Maastricht, the Netherlands

ARTICLE INFO

*Keywords:*
COPD
Lung emphysema
LPS
Non-digestible oligosaccharides

ABSTRACT

Chronic obstructive pulmonary disease (COPD), a progressive inflammatory disorder, is also known as a systemic inflammatory disease, in which the gut-lung interaction plays an important role. The use of non-digestible oligosaccharides (NDOs) has gained attention in airway diseases due to their systemic effects on inflammatory markers. Here, the preventive effects of specific non-digestible oligosaccharides (GOS/lcFOS/lvPectin) were investigated in a murine lipopolysaccharide (LPS)-induced emphysema model. Nasal LPS-installations were used to induce emphysema in male BALB/c mice. Two weeks prior to the first LPS challenge, mice received GOS/lcFOS/lvPectin (9:1:2) mixture by gavage (25 mg NDOs/200 µl PBS) five days a week until day 28. The LPS-induced neutrophil influx in bronchoalveolar lavage fluid (BALF) decreased by > 60% after intervention with GOS/lcFOS/lvPectin and the development of lung emphysema, measured by mean linear intercept, was prevented. Macroscopic examination of heart tissue revealed that GOS/lcFOS/lvPectin pretreatment attenuated the LPS-induced increase in right ventricular heart hypertrophy. In summary, GOS/lcFOS/lvPectin prevented characteristic features of COPD in the LPS-induced lung emphysema model. Since no therapy is available to stop or prevent development of COPD, oligosaccharides may have potential to be used as stand alone or in combination with other anti-inflammatory nutrients or drugs to diminish disease progression in COPD.

## 1. Introduction

Chronic obstructive pulmonary disease (COPD) is a common and progressive respiratory disorder characterized by persistent respiratory symptoms, airflow limitation, chronic bronchitis and emphysema. According to the World health organization (WHO), COPD was the main cause of worldwide morbidity and mortality in 2012 and by 2020 it is estimated to rank third in worldwide mortality [1]. The primary cause of COPD is first and secondhand smoking (including passive exposure). LPS is present in high concentrations in tobacco and can be partly attributed to the emphysema-like changes [2]. Other risk factors may include indoor and outdoor air pollution, and frequent lower respiratory tract infections during childhood.

Smoking-induced inflammatory reactions in the airway lead to the recruitment of inflammatory cells such as, neutrophils, macrophages, T lymphocytes and dendritic cells. These inflammatory cells release proteases, reactive oxygen species, chemokines and cytokines. This leads to the destruction of alveolar walls accompanied by airway remodeling processes, resulting in a loss of airway functional characteristic of pulmonary emphysema [3,4].

Accumulating evidence reveals that lung inflammation and airway remodeling are not the only manifestations of COPD, and in recent years COPD has been recognized as a systemic inflammatory disease [5–7]. Exactly how and why COPD patients develop systemic inflammation is not known yet. One potential explanation could be that airway inflammatory processes spill over into the systemic circulation leading to systemic inflammatory reactions. It is also possible that inhaled toxic substrates enter the circulation and induce systemic inflammatory responses leading to comorbidities, such as vascular disease [8,9]. Another explanation might be a disturbed intestinal integrity often found in patients with COPD. Compared to healthy elderly subjects, COPD patients show increased levels of markers for intestinal

* Corresponding author at: Utrecht University, Department of Pharmaceutical Sciences, Division of Pharmacology, Universiteitsweg 99, 3584 CG, Utrecht, the Netherlands.
*E-mail address:* S.Braber@uu.nl (S. Braber).

https://doi.org/10.1016/j.phanu.2019.100163
Received 15 July 2019; Received in revised form 31 July 2019; Accepted 3 August 2019
Available online 05 August 2019
2213-4344/ © 2019 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).

permeability at rest, which were even enhanced during activities of daily living [10]. Moreover, markers of disturbed intestinal integrity were even further enhanced in COPD patients suffering acute exacerbations compared to stable COPD patients [11]. Both hypoxic damage and circulating inflammatory mediators are thought to contribute to the loss of epithelial integrity due to alterations in tight junctions leading to a reduced enterocyte interconnectivity and increased intestinal permeability [12].

Accumulating data indicate that dietary fiber intake play a role in the incidence of COPD. A low dietary fiber intake is associated with a reduction in lung function [13], while a high-fiber diet can reduce the risk of COPD incidence [14,15]. The precise mechanism of action is still largely unknown, but most likely it is a combination of the stimulation of beneficial bacteria in the gut, the production of fermentation-derived short-chain fatty acids (SCFA) and the direct effects on intestinal epithelial and immune cells [15]. Regardless, intake of dietary fibers is associated with a reduction in inflammatory markers systemically, but also locally in the lungs [16,17].

Various lines of research have shown that specific fibers, including non-digestible oligosaccharides (NDOs), can have immunomodulatory properties in various diseases and conditions, such as allergy, infections, cancer and HIV [18–22]. Other studies using a mixture of different types of oligosaccharides, including galacto-oligosaccharides (GOS), fructo-oligosaccharides (FOS) and pectin-derived acidic oligosaccharides (pAOS) have shown possible beneficial effects on the infant's immune system [23,24]. GOS, oligosaccharides based on the milk sugar lactose, are widely used in infant formulas and resemble oligosaccharides that occur naturally in human breast milk [25]. FOS, including fructose oligomers, can be obtained from many plants and vegetables, such as chicory [26]. GOS and FOS are of interest due to their prebiotic and potential immunomodulatory effects [27]. Pectin is the source of POS in natural products, such as citrus and apple peel, pulp from potato, sugar beets and carrots and other sources [28]. Fermentation experiments show that pectin is not degraded in the small intestine, but more or less completely fermented by the colonic microbiota in a multistep process. During this fermentation process of pectin, consisting of chains of α-1,4 galacturonic acid, POS monomers are formed [29]. In literature, POS monomers are often referred to as pAOS, which have been found to have immunomodulatory properties [20]. A diet containing short-chain galacto-oligosaccharides (GOS), long-chain fructo-oligosaccharides (lcFOS) and pAOS was shown to reduce airway inflammation and hyperresponsiveness following an antigen challenge in a mouse model of allergic asthma [30]. Similar results were obtained in a mouse model of cow's milk allergy. Here, a mixture of GOS, lcFOS and pAOS was shown to reduce the allergic effector response via induction of the anti-inflammatory components interleukin-10 (IL-10), transforming growth factor β (TGFβ) and T-regulatory cells (Treg) [31]. In addition, POS have been shown to have anti-inflammatory and antioxidant properties which can be considered health promoting functions [32].

These findings lead us to consider a dietary intervention with specific non-digestible oligosaccharides in a mouse model of COPD [33]. We hypothesize that supplementation of a diet with GOS, lcFOS and low viscosity pectin (lvPectin) would reduce LPS-induced lung inflammation and by doing so, ameliorate inflammation-associated emphysema.

## 2. Materials and methods

### 2.1. Animals

Male BALB/c by JIco mice (6 weeks old) of specific pathogen free quality were obtained from Charles River Laboratories, Someren, the Netherlands. The mice were housed in groups and kept in two cohorts in macrolon cages (6 mice/cage) with sawdust bedding. Mice were held in a light/dark cycle of 12 h/12 h, controlled relative humidity and temperature with ad libitum access to tap water and pelleted food. Upon arrival, all mice were randomly allocated to the control and experimental groups: control group with PBS gavages, PBS group with GOS/lcFOS/lvPectin gavages, LPS group with PBS gavages, LPS group with GOS/lcFOS/lvPectin gavages (n = 5–12).

All experimental procedures were approved by the Animal Experiments Committee (DEC-consult, Utrecht, The Netherlands) and complied with the principles of good laboratory animal care following the EU-directive for the protection of animals used for scientific purposes.

### 2.2. Dietary interventions

All mice were fed standard rodent diet and were acclimatized for 7 days. Fourteen days prior to the first PBS or LPS administration (day -14), a GOS:lcFOS:lvPectin mixture (9:1:2) with GOS prepared from lactose (Friesland Campina, Amersfoort, the Netherlands), lcFOS isolated from chicory (Orafti, Wijchen, the Netherlands) and lvPectin containing 88% non-digestible dietary fibers isolated from apple (Herbapekt SF-50-A-LV, HERBSTREITH & FOX, Neuenburg, Germany) or PBS (control) was administered orally via gavage (25 mg NDOs in 200 µl PBS) 5 days per week till day 28 (Fig. 1).

### 2.3. LPS-induced lung emphysema

LPS from *Escherichia coli* serotype 055:B5 was obtained from Sigma-Aldrich (St. Louis, MO). Mice (n = 12/group) were anesthetized by isoflurane inhalation and LPS (5 µg in 50 µl PBS/mouse) was intranasally administered to the mice twice a week for 4 weeks (day 0, 3, 7, 10, 14, 17, 21 and 24) to induce lung emphysema (Fig. 1), while the control mice received intranasally PBS (50 µl/mouse) [33]. At day 28 mice were euthanized by intraperitoneal pentobarbital overdose (Nembutal, Ceva Santé Animale, Naaldwijk, the Netherlands).

### 2.4. Growth pattern

Body weight of the individual animals was recorded once a week (day –14, -7, 0, 7, 14, 21, 28). Results are presented as growth (%). Growth (%) was calculated as:

[(body weight day X - body weight day -14)/body weight day -14] * 100%; X= -7, 0, 7, 14, 21, 28.

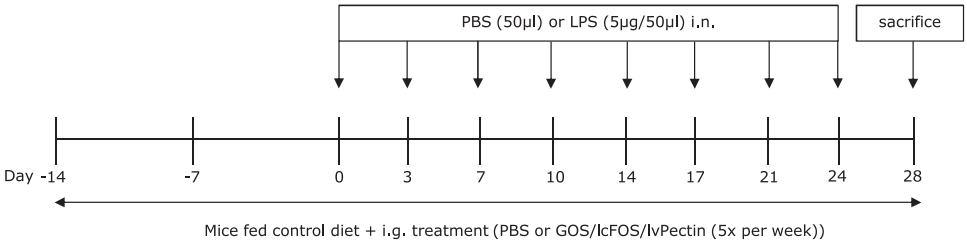


**Fig. 1.** Time schedule of the LPS-induced emphysema model. PBS or LPS was intranasally administered to the male BALB/c mice twice a week for 4 weeks (day 0, 3, 7, 10, 14, 17, 21 and 24). Fourteen days prior to the first PBS or LPS administration, a mixture with PBS or GOS/lcFOS/lvPectin (9:1:2 ratio) was administered orally via gavage (5 days/week). All mice were sacrificed on day 28.

### 2.5. Bronchoalveolar lavage (BAL)

After euthanasia, a cannula was inserted into the trachea and lungs were lavaged 4 times with 1 mL pyrogen-free saline (0.9% NaCl, 37 °C) *in situ*. The BAL fluid was centrifuged (400 × *g*, 5 min at 4 °C), and total numbers of BAL fluid cells were counted under light microscopy using a Bürker-Türk chamber (magnification 100×). Differential BAL fluid cell counts were conducted on cytospin preparations stained with DiffQuik™ (Merz & Dade A.G., Düdingen, Switzerland) and cells were identified as macrophages, lymphocytes, and neutrophils according to standard morphology. At least 200 cells per cytospin preparation were counted, and the absolute number of each cell type was calculated [22].

### 2.6. Mean linear intercept ($L_m$)

A separate group of mice was used to analyze lung emphysema by assessing the $L_m$ on lung sections after euthanasia. Briefly, mice were exsanguinated by cutting the caudal vena cava to prevent the blood flow into the bases of the lungs. A cannula was inserted into the trachea, lungs and heart were removed en bloc, and lungs were inflated via the cannula by gentle infusion of 10% formalin (Merck, Darmstadt, Germany) at a constant fluid pressure of 25 cm for 5 min. Lungs in 10% formalin were immersed for at least 24 h. The right lung was embedded in paraffin (Stemcowax, Adamas Instruments, Rhenen, the Netherlands) after dehydration in graded ethanol series followed by xylene [34].

Subsequently, sections of 5 µm were cut at 200, 400, 600, and 800 µm in dorsal ventral plane. The sections were placed on poly-l-lysine-coated glass slides and deparaffinized using xylene and ascending ethanol series. All sections were stained with hematoxylin & eosin according to standard methods and were dehydrated in graded ethanol concentrations and xylene before being coverslipped with DePeX (Serva, Heidelberg, Germany).

Morphometric assessment of emphysema, included determination of the average interalveolar distance, was estimated by the $L_m$ analysis. $L_m$ is the most common morphometric method to assess lung emphysema in animal models [35–38]. The $L_m$ was determined by light microscopy at a total magnification of ×100, whereby 24 random photomicroscopic images per lung tissue section (6 images per depth) were evaluated by microscopic projection onto a reference grid. By dividing total grid length by the number of alveolar wall-grid line intersections, the $L_m$ (in µm) was calculated.

### 2.7. Right ventricular heart hypertrophy

The heart was isolated, and the right ventricle (RV) was completely separated and removed from the lower heart with use of a dissecting microscope. The RV and left ventricle plus septum (LV + S) were weighed separately after having been blotted dry. The ratio of RV to LV + S weight was used as index of right ventricular hypertrophy.

### 2.8. Statistical analysis

Unless stated otherwise, data are expressed as arithmetic average ± standard error of mean and comparisons between groups were made using a One-Way-ANOVA and post hoc Tukey multiple comparisons test. A probability value $P < 0.05$ was considered significant.

## 3. Results

### 3.1. GOS/lcFOS/lvPectin mixture regulates the LPS-induced weight loss in mice

The body weights of all animals were determined once a week throughout the experiment. As depicted in Fig. 2A, LPS-treated group gained significantly less body weight compared to the control group. The difference in body weight gain between LPS and PBS-treated animals seemed to be less when the LPS-treated animals receive the GOS/lcFOS/lvPectin mixture ($P = 0.052$). Lines of individual groups were summarized as Areas-Under-Curve (AUC) and this summary measure was used for statistics (Fig. 2B).

### 3.2. GOS/lcFOS/lvPectin mixture inhibits the LPS-induced increase in neutrophils in the BALF

To evaluate the immunological response in the lungs, total BAL cell count and macrophages, neutrophils and lympocytes were determined. The total BAL cells and the amount of macrophages, neutrophils and lymphocytes are increased in the LPS-exposed mice (Fig. 3A-D). The increase in neutrophils in the LPS group tends to be diminished ($P = 0.058$) by administration of the GOS/lcFOS/lvPectin mixture and is significantly lowered when calculated as % of neutrophils from total BALF cells ($P = 0.048$). No significant effect on the macrophages, lymphocytes and total BAL cells was observed after GOS/lcFOS/lvPectin treatment.

### 3.3. GOS/lcFOS/lvPectin mixture prevents the LPS-induced increase in $L_m$

The mean linear intercept, commonly used to quantify lung emphysema, is significantly higher in the LPS-exposed animals compared to the PBS-exposed animals with or without GOS/lcFOS/lvPectin treatment (Fig. 4A). This higher level of $L_m$ is significantly and completely prevented in the LPS-exposed mice that receive gavages with GOS/lcFOS/lvPectin (Fig. 4A). Representative pictures from the $L_m$ measurement in the PBS-treated mice (Fig. 4B), the LPS-treated mice (Fig. 4C) and the LPS-treated mice in combination with administration of GOS/lcFOS/lvPectin (Fig. 4D) are depicted.

### 3.4. GOS/lcFOS/lvPectin mixture reduces the LPS-induced right ventricle hypertrophy

Right ventricle heart hypertrophy was measured as an indication of the development of lung emphysema. The LPS administration in this lung emphysema model tends to cause right ventricle heart hypertrophy as shown in Fig. 5. The GOS/lcFOS/lvPectin mixture significantly reduces the right ventricle heart hypertrophy.

## 4. Discussion

Chronic exposure to cigarette smoke in humans is associated with the development of emphysema and chronic bronchitis. The pulmonary inflammation and airway obstruction observed in smokers and COPD patient could partially be attributed to the responses to LPS, which is present in high concentrations in tobacco [39]. In this study, mice were exposed to LPS by repetitive intranasal instillation during 4 weeks, and this murine model showed similar characteristics as observed in COPD patients.

In the present study, mice instilled with LPS showed a significant decrease in body weight gain compared to the control group, which is in line with other *in vivo* studies, where mice in different lung emphysema models demonstrated a significant reduction in body weight gain [40,41].

The number of inflammatory cells in BALF, including macrophages, lymphocytes and neutrophils, were higher in the LPS-exposed mice, as also observed by other authors [42–47]. The mean linear intercept, a measure to quantify emphysema, was significantly higher after LPS instillation. Previous studies demonstrated that chronic treatment with LPS causes parenchymal architectural changes in the lung [48,49]. Pulmonary hypertension is a common complication of COPD and may result in alterations in structure and function of the right ventricle of the heart. In this study, right ventricle heart hypertrophy was detected in the LPS-exposed mice, which was also shown in a guinea pig model

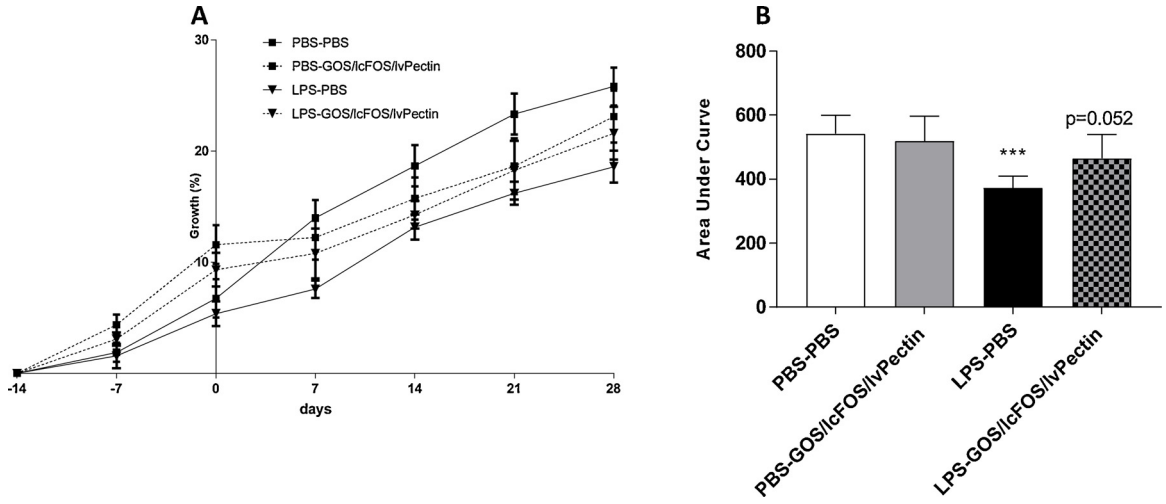


**Fig. 2.** GOS/lcFOS/lvPectin mixture regulates the LPS-induced weight loss in mice.
Mice received a gavage with PBS or GOS/lcFOS/lvPectin (day -14 till day 28, 5 days/week) and were exposed to PBS or LPS twice a week for 4 weeks. Body weights were determined once a week throughout the experiment. Values are expressed in % growth (body weight day X - body weight day -14)/body weight (-14) * 100%) as mean ± SEM (A). Areas-Under-Curve (AUC) (in % growth) were calculated for statistical analysis as mean ± SEM (B). ***P < 0.001; significantly different from control group, n = 9–12 mice/group.

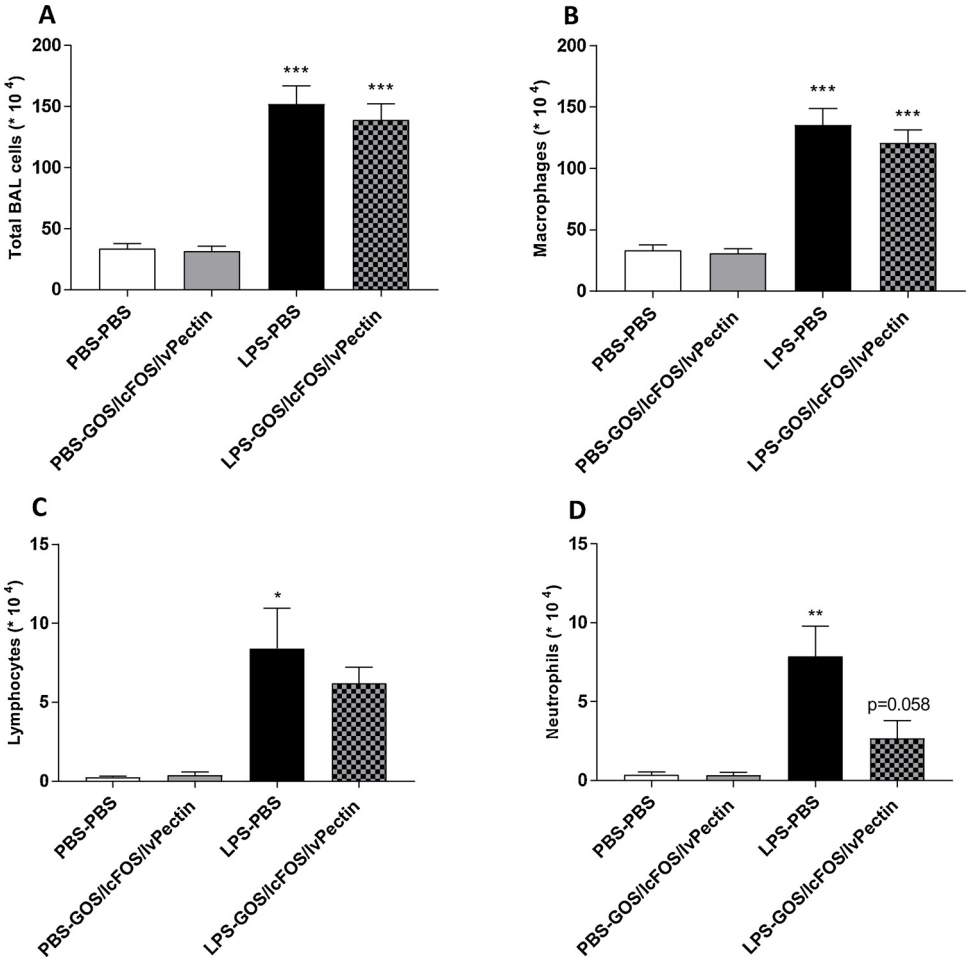


**Fig. 3.** GOS/lcFOS/lvPectin mixture inhibits the LPS-induced increase in neutrophils in the BALF.
Mice received a gavage with PBS or GOS/lcFOS/lvPectin (day -14 till day 28, 5 days/week) and were exposed to PBS or LPS twice a week for 4 weeks. At day 28 lungs were lavaged and BALF was collected for total (A) and differential BAL cell counts, including macrophages (B), lymphocytes (C) and neutrophils (D). Values are expressed as mean ($x10^4$) ± SEM. **P < 0.01, ***P < 0.001; significantly different from control group, P = 0.058; compared to LPS group, n = 6–10 mice/group.

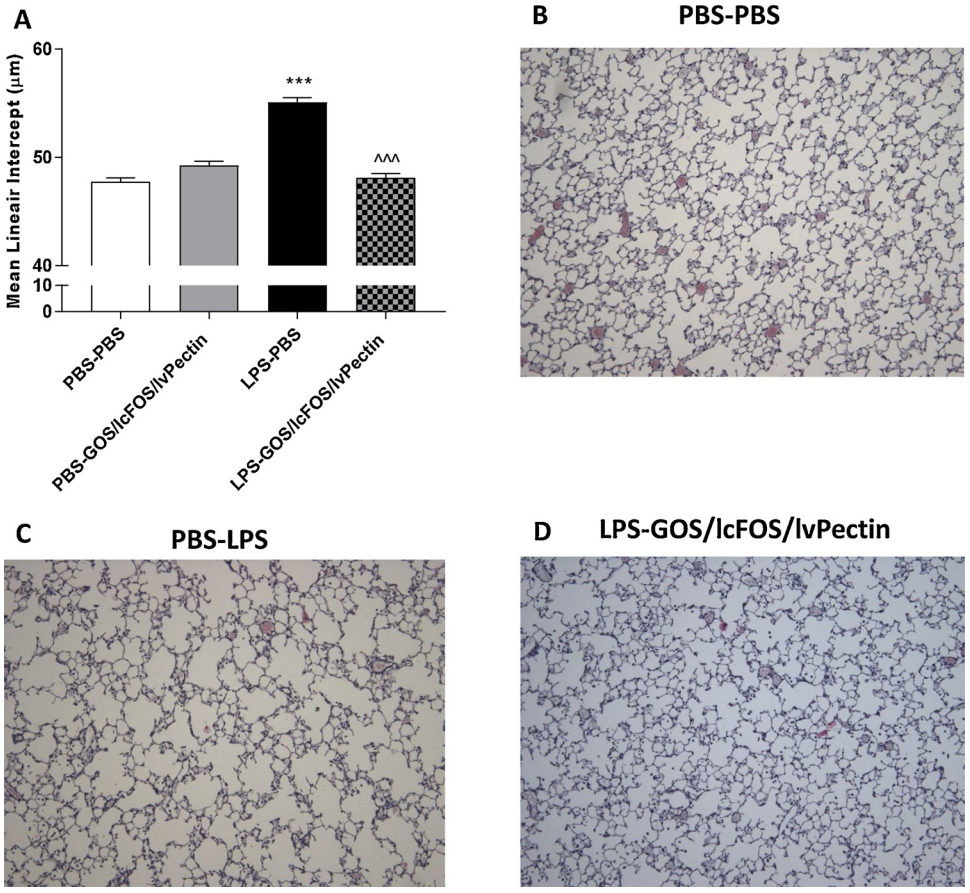


**Fig. 4.** GOS/lcFOS/lvPectin mixture prevents the LPS-induced increase in $L_m$.
Mice received a gavage with PBS or GOS/lcFOS/lvPectin (day -14 till day 28, 5 days/week) and were exposed to PBS or LPS twice a week for 4 weeks. At day 28 lungs were fixed in 10% formalin, embedded in paraffin and morphometric assessment of emphysema was determined by $L_m$ analysis on H&E stained lung sections. Values are expressed as mean (µm) ± SEM (A) with representative pictures of H&E stained lung tissue from control group (B), LPS group (C) and LPS group in combination with administration of GOS/lcFOS/lvPectin (D). ***P < 0.001; significantly different from control group, ^^^P < 0.001; significantly different from LPS group, n = 5 mice/group.

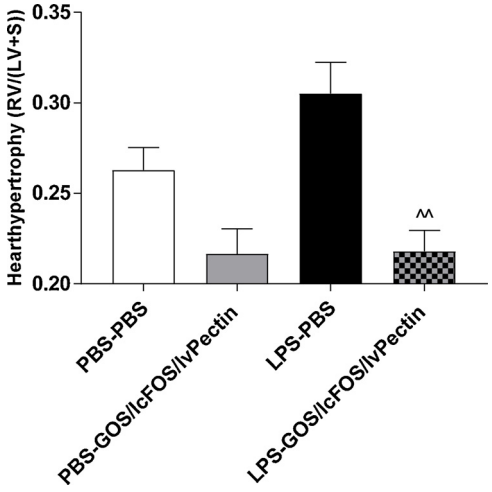


**Fig. 5.** GOS/lcFOS/lvPectin mixture reduces the LPS-induced right ventricle hypertrophy.
Mice received a gavage with PBS or GOS/lcFOS/lvPectin (day -14 till day 28, 5 days/week) and were exposed to PBS or LPS twice a week for 4 weeks. At day 28, the heart was isolated and the ratio of right ventricle weight to left ventricle + septum weight (RV/(LV + S) was used as index of right ventricular hypertrophy. Values are expressed as mean ± SEM. ^^P < 0.01; significantly different from LPS group, n = 6–10 mice/group.

after repeated LPS exposure [50]. This LPS-induced emphysema mouse model was designed to assess the effects of NDO supplementation on modulating inflammatory responses, as well as emphysematous phenotypes observed in COPD.

Some specific NDOs are known as functional food ingredients, which promote the growth of beneficial bacteria in the gut and contain gut health-promoting properties [51]. Besides the metabolic function of the gut microbiota, the microbiota provides crucial signals for the development and function of the immune system [52,53]. There is a vital cross talk between the gastrointestinal tract and the respiratory system, which are part of a shared mucosal immune system, called the gut–lung axis [54].

In the present study, GOS/lcFOS/lvPectin supplementation decreased the neutrophil numbers in the BALF, whereas the lymphocyte and macrophage numbers in the BALF were not affected. Verheijden et al. [55,56] showed that dietary intervention with GOS or synbiotics decreased the eosinophil numbers in the BALF in a murine house dust mite-induced asthma model. Another study reported that dietary pAOS improved the outcome of a *Pseudomonas aeruginosa* infection in the lung by modulating the inflammatory and immune responses [20]. In addition, the findings of Schijf et al. [57] indicate that prophylactic dietary intervention with GOS/lcFOS/pAOS can increase Th1 responses and reduce the Th2 responses in the lung of respiratory syncytial virus infected mice. Furthermore, feed supplementation with 1% GOS/lcFOS/pAOS mixture in a preventive protocol, was able to reduce inflammatory cells in BALF following ovalbumin sensitization in murine allergy model [30].

To our knowledge, there is no literature available about the effect of non-digestible oligosaccharides on the alveolar wall destruction and the development of cardiac hypertrophy. Hence, this is the first article describing the beneficial effect of this specific NDO mixture on Lm measurement and RV hypertrophy.

The underlying mechanism how these NDOs influence the lung environment via oral application is still not entirely understood. First of all, the gut microbiota interacts with the host immune system. NDOs modulate the microbial composition and function, which have been linked to alterations in immune responses in both the gut and the lungs, confirming an immunological link between the gut and the lungs. One

possibility is that microbiota can affect the mucosal and systemic immune responses by the production of a wide range of metabolites, such as short chain fatty acids (SCFA), produced upon bacterial fermentation of NDOs. [58–60]. It is known that circulating SCFA can attenuate airway inflammation via GPR41 and GPR43 receptors [61].

Besides the microbiota-dependent effects of non-digestible oligosaccharides, results from different *in vitro* studies from our group indicated that galacto-oligosaccharides can also directly protect the intestinal epithelial barrier against different stressors [62–64]. Interestingly, in a recent publication, Rutten et al. [65] showed that the intestinal integrity is disturbed in patients with COPD. Moreover, these patients also showed increased small intestinal injury measured by plasma concentrations of intestinal fatty-acid binding protein during their activities of daily living. The increased intestinal permeability and intestinal injury in COPD patients allows the paracellular infiltration of pathogens, antigens and proinflammatory substances, which is supposed to induce local or systemic inflammation [66].

In addition, even systemic effects of oligosaccharides have been predicted, since Gnoth et al. [67] and Eiwegger et al. [68] showed *in vitro* evidence for transport of human milk oligosaccharides and prebiotic NDOs, like GOS, across the intestinal epithelial layer. These findings indicate that orally applied oligosaccharides are absorbed, which was confirmed by the presence of (human) milk oligosaccharides in the circulation (blood and urine) of breastfed infants [69] and piglets fed a milk replacer supplemented with GOS [70]. When these NDOs can reach the lungs, they could directly stimulate the airway microbiota, which may affect the local immune cells and consequently modulates the immune responses in the lungs.

## 5. Conclusions

Although there are drug treatments for COPD to control symptoms, maximize pulmonary function and reduce exacerbation rates, none of the available drug therapies reduce the progression of the disease. New approaches that control the inflammatory and destructive processes in the lung are urgently needed. Hence, the use of specific NDOs might be an interesting therapeutic option for COPD patients by targeting the gut-lung axis and affecting both pulmonary as well as extrapulmonary effects. We have shown for the first time that pretreatment with the specific NDO mixture of GOS/lcFOS/lvPectin effectively prevented alveolar wall destruction, right ventricle hypertrophy and neutrophil infiltration into the lungs after LPS instillation. NDOs may be potential candidates to prevent or treat (LPS-induced) COPD in the future. However, further research is needed to elucidate the mechanisms by which these oligosaccharides exert their beneficial effect on the lungs.

## Animal experiments

The mice experiments described in the article have been carried out in accordance with EU directive for animal experiments.

## Declaration of Competing Interest

HJ: No relevant conflicts of interest
JvB: Employed by Danone Nutricia Research
KV: No relevant conflicts of interest
TL: No relevant conflicts of interest
AvH: Employed by Danone Nutricia Research
JG: Employed by Danone Nutricia Research
GF: No relevant conflicts of interest
SB: No relevant conflicts of interest

## Acknowledgements

This work was supported by a LSH-TKI Lung Foundation grant 10.2.16.119, 2017, the Netherlands, entitled ‘Food for thought and active lifestyle in COPD’.

## References

[1] C.D. Mathers, D. Loncar, Projections of Global Mortality and Burden of Disease from 2002 to 2030, PLoS Med. 3 (2006) e442, https://doi.org/10.1371/journal.pmed.0030442.
[2] L. Larsson, C. Pehrson, T. Dechen, M. Crane-Godreau, Microbiological components in mainstream and sidestream cigarette smoke, Tob. Induc. Dis. 10 (2012) 13, https://doi.org/10.1186/1617-9625-10-13.
[3] P.J. Barnes, Cellular and molecular mechanisms of asthma and COPD, Clin. Sci. 131 (2017) 1541 LP–1558, https://doi.org/10.1042/CS20160487.
[4] K.F. Chung, I.M. Adcock, Multifaceted mechanisms in COPD: inflammation, immunity, and tissue repair and destruction, Eur. Respir. J. 31 (2008) 1334 LP–1356, https://doi.org/10.1183/09031936.00018908.
[5] J.R. Hurst, W.R. Perera, T.M.A. Wilkinson, G.C. Donaldson, J.A. Wedzicha, Systemic and upper and lower airway inflammation at exacerbation of chronic obstructive pulmonary disease, Am. J. Respir. Crit. Care Med. 173 (2006) 71–78, https://doi.org/10.1164/rccm.200505-704OC.
[6] D.D.S.W.Q. Gan, S.F.P. Man, A. Senthilselvan, Association between chronic obstructive pulmonary disease and systemic inflammation: a systematic review and a meta- analysis, Thorax 59 (2004) 574–580, https://doi.org/10.1136/thx.2003.019588.
[7] C.E. Pelgrim, J.D. Peterson, H.R. Gosker, A.M.W.J. Schols, A. van Helvoort, J. Garssen, G. Folkerts, A.D. Kraneveld, Psychological co-morbidities in COPD: Targeting systemic inflammation, a benefit for both? Eur. J. Pharmacol. 842 (2019) 99–110, https://doi.org/10.1016/j.ejphar.2018.10.001.
[8] M.R. Miller, J.B. Raftis, J.P. Langrish, S.G. Mclean, P. Samutrtai, S.P. Connell, S. Wilson, A.T. Vesey, P.H.B. Fokkens, A.J.F. Boere, P. Krystek, C.J. Campbell, P.W.F. Hadoke, K. Donaldson, F.R. Cassee, D.E. Newby, R. Du, N.L. Mills, Inhaled nanoparticles accumulate at sites of, ACS Nano 176 (2017) 532–555, https://doi.org/10.1021/acsnano.6b08551.
[9] T.K. Alicic, R. Patakoti, Direct and indirect effects of PM on the cardiovascular system, Adv. Chronic Kidney Dis. 208 (2013) 293–299, https://doi.org/10.1016/j.toxlet.2011.11.008.Direct.
[10] E.P.A. Rutten, K. Lenaerts, W.A. Buurman, Effects of activities of daily living with COPD, Chest 145 (2014) 245–252, https://doi.org/10.1378/chest.13-0584.
[11] G.G.U.R. Roy, T.M. Sprooten, I. Grimbergen, D. Braeken, K. Lenaerts, E. Rutten, E.F.M. Wouters, Increased small intestinal permeability during severe acute exacerbations of, Respiration 95 (2018) 334–342, https://doi.org/10.1159/000485935.
[12] J.R. Turner, Intestinal mucosal barrier function in health and disease, Nat. Rev. Immunol. 9 (2009) 799–809, https://doi.org/10.1038/nri2653.
[13] R. Young, The relationship between dietary Fiber intake and lung function in the national health and nutrition examination surveys, Ann. Am. Thorac. Soc. 13 (2016) 643–650, https://doi.org/10.1513/AnnalsATS.201509-609OC.
[14] J. Kaluza, H. Harris, A. Wallin, A. Linden, A. Wolk, Dietary fiber intake and risk of chronic obstructive pulmonary disease, Epidemiology 29 (2018) 254–260, https://doi.org/10.1097/EDE.0000000000000750.
[15] S.A. Fonseca, E. Wald, B. Van Den Borst, H. Gosker, Dietary fibre and fatty acids in chronic obstructive pulmonary disease risk and progression: a systematic review, Respirology 19 (2014) 176–184, https://doi.org/10.1111/resp.12229.
[16] Y. Ma, J.A. Griffith, L. Chasan-taber, B.C. Olendzki, E. Jackson, E.J.S. Iii, W. Li, S.L. Pagoto, A.R. Hafner, I.S. Ockene, Association between dietary fiber and serum C-reactive protein 1 – 3, Am. J. Clin. Nutr. 83 (2006) 760–766.
[17] K.N. Grooms, M.J. Ommerborn, Q. Pham, L. Djoussé, Dietary Fiber intake and cardiometabolic risks among US adults, NHANES 1999-2010, Am. J. Med. 126 (2013) 1059–1067, https://doi.org/10.1016/j.amjmed.2013.07.023 e4.
[18] P. Cahn, K. Ruxrungtham, B. Gazzard, R.S. Diaz, A. Gori, D.P. Kotler, A. Vriesema, N.A. Georgiou, J. Garssen, M. Clerici, J.M.A. Lange, for the B. (Blinded N.S. for I, T.E.S. Team, The immunomodulatory nutritional intervention NR100157 reduced CD4+ T-Cell decline and immune activation: a 1-Year multicenter randomized controlled double-blind trial in HIV-Infected persons not receiving antiretroviral therapy (The BITE study), Clin. Infect. Dis. 57 (2013) 139–146, https://doi.org/10.1093/cid/cit171.
[19] J. Faber, P. Vos, D. Kegler, K. van Norren, J.M. Argilés, A. Laviano, J. Garssen, A. van Helvoort, Beneficial immune modulatory effects of a specific nutritional combination in a murine model for cancer cachexia, Br. J. Cancer 99 (2008) 2029, https://doi.org/10.1038/sj.bjc.6604785.
[20] H. Bernard, J.-L. Desseyn, F. Gottrand, B. Stahl, N. Bartke, M.-O. Husson, Pectin-derived acidic oligosaccharides improve the outcome of Pseudomonas aeruginosa lung infection in C57BL/6 mice, PLoS One 10 (2015) e0139686, , https://doi.org/10.1371/journal.pone.0139686.
[21] J. Faber, K. van Limpt, D. Kegler, Y. Luiking, J. Garssen, A. van Helvoort, A.P. Vos, J. Knol, Bacterial translocation is reduced by a specific nutritional combination in mice with chemotherapy-induced neutropenia, J. Nutr. 141 (2011) 1292–1298, https://doi.org/10.3945/jn.110.136986.
[22] K.A.T. Verheijden, L.E.M. Willemsen, S. Braber, T. Leusink-Muis, D.J.M. Delsing, J. Garssen, A.D. Kraneveld, G. Folkerts, Dietary galacto-oligosaccharides prevent airway eosinophilia and hyperresponsiveness in a murine house dust mite-induced asthma model, Respir. Res. 16 (2015) 1–9, https://doi.org/10.1186/s12931-015-0171-0.
[23] E.A.M. Westerbeek, R.M. van Elburg, A. van den Berg, J. van den Berg, J.W.R. Twisk, W.P.F. Fetter, H.N. Lafeber, Design of a randomised controlled trial on immune effects of acidic and neutral oligosaccharides in the nutrition of preterm

infants: carrot study, BMC Pediatr. 8 (2008) 46, https://doi.org/10.1186/1471-2431-8-46.
[24] A.P. Vos, M. Haarman, J.-W.H. VanGinkel, J. Knol, J. Garssen, B. Stahl, G. Boehm, L. M'Rabet, Dietary supplementation of neutral and acidic oligosaccharides enhances Th1-dependent vaccination responses in mice, Pediatr. Allergy Immunol. 18 (2007) 304–312, https://doi.org/10.1111/j.1399-3038.2007.00515.x.
[25] D.P.M. Torres, M. do P.F. Gonçalves, J.A. Teixeira, L.R. Rodrigues, Galacto-oligosaccharides: production, properties, applications, and significance as prebiotics, Compr. Rev. Food Sci. Food Saf. 9 (2010) 438–454, https://doi.org/10.1111/j.1541-4337.2010.00119.x.
[26] D.A. Flores-Maltos, S.I. Mussatto, J.C. Contreras-Esquivel, R. Rodríguez-Herrera, J.A. Teixeira, C.N. Aguilar, Biotechnological production and application of fructooligosaccharides, Crit. Rev. Biotechnol. 36 (2016) 259–267, https://doi.org/10.3109/07388551.2014.953443.
[27] P.V. Jeurink, A. Rijnierse, J. Garssen, Mechanisms underlying immune effects of dietary oligosaccharides 1–4, Am. J. Clin. Nutr. 98 (2013) 572–577, https://doi.org/10.3945/ajcn.112.038596.572S.
[28] F. Dranca, M. Oroian, Extraction, purification and characterization of pectin from alternative sources with potential technological applications, Food Res. Int. 113 (2018) 327–350, https://doi.org/10.1016/j.foodres.2018.06.065.
[29] G. Dongowski, A. Lorenz, J. Proll, The degree of methylation influences the degradation of pectin in the intestinal tract of rats and in vitro, J. Nutr. 132 (2002) 1935–1944, https://doi.org/10.1093/jn/132.7.1935.
[30] A.P. Vos, B.C. Van Esch, B. Stahl, L.M. Rabet, G. Folkerts, F.P. Nijkamp, J. Garssen, Dietary supplementation with specific oligosaccharide mixtures decreases parameters of allergic asthma in mice, Int. Immunopharmacol. 7 (2007) 1582–1587, https://doi.org/10.1016/j.intimp.2007.07.024.
[31] J. Kerperien, D. Veening-griffioen, T. Wehkamp, B.C.A.M. Van Esch, G.A. Hofman, P. Cornelissen, L. Boon, P.V. Jeurink, J. Garssen, L.M.J. Knippels, L.E.M. Willemsen, IL-10 receptor or TGF- β neutralization abrogates the protective effect of a specific nondigestible oligosaccharide mixture in cow-milk-allergic mice, J. Nutr. Nutr. Immunol. 148 (2018) 1372–1379, https://doi.org/10.1093/jn/nxy104.
[32] H. Tan, W. Chen, Q. Liu, G. Yang, K. Li, Pectin oligosaccharides ameliorate colon cancer by regulating oxidative stress- and inflammation-activated signaling pathways, Front. Immunol. 9 (2018) 1504 https://www.frontiersin.org/article/10.3389/fimmu.2018.01504.
[33] A.H. Van Houwelingen, N.M. Weathington, V. Verweij, J.E. Blalock, F.P. Nijkamp, G. Folkerts, Induction of lung emphysema is prevented by L -arginine-threonine-arginine, FASEB J. 22 (2008) 3403–3408, https://doi.org/10.1096/fj.07-096230.
[34] S. Braber, P.A.J. Henricks, F.P. Nijkamp, A.D. Kraneveld, G. Folkerts, Inflammatory changes in the airways of mice caused by cigarette smoke exposure are only partially reversed after smoking cessation, Respir. Res. 11 (2010) 99, https://doi.org/10.1186/1465-9921-11-99.
[35] D.M. Brass, J.W. Hollingsworth, M. Cinque, Z. Li, E. Potts, E. Toloza, W.M. Foster, D.A. Schwartz, Chronic LPS inhalation causes emphysema-like changes in mouse lung that are associated with apoptosis, Am. J. Respir. Cell Mol. Biol. 39 (2008) 584–590, https://doi.org/10.1165/rcmb.2007-0448OC.
[36] S. Braber, K.A.T. Verheijden, P.A.J. Henricks, A.D. Kraneveld, G. Folkerts, A comparison of fixation methods on lung morphology in a murine model of emphysema, Am. J. Physiol. Cell. Mol. Physiol. 299 (2010) L843–L851, https://doi.org/10.1152/ajplung.00192.2010.
[37] M.P. Andersen, A.R. Parham, J.C. Waldrep, W.N. McKenzie, R. Dhand, Alveolar fractal box dimension inversely correlates with mean linear intercept in mice with elastase-induced emphysema, Int. J. Chron. Obstruct. Pulmon. Dis. 7 (2012) 235–243, https://doi.org/10.2147/COPD.S26493.
[38] M.S. Dunnill, Quantitative methods in the study of pulmonary pathology, Thorax 17 (1962) 320–328, https://doi.org/10.1136/thx.17.4.320.
[39] B. Szponar, C. Pehrson, L. Larsson, Bacterial and fungal markers in tobacco smoke, Sci. Total Environ. 438 (2012) 447–451, https://doi.org/10.1016/j.scitotenv.2012.08.067.
[40] L. Lüthje, T. Raupach, H. Michels, B. Unsöld, G. Hasenfuss, H. Kögler, S. Andreas, Exercise intolerance and systemic manifestations of pulmonary emphysema in a mouse model, Respir. Res. 10 (2009) 7, https://doi.org/10.1186/1465-9921-10-7.
[41] M. Rinaldi, K. Maes, S. De Vleeschauwer, D. Thomas, E.K. Verbeken, M. Decramer, W. Janssens, G.N. Gayan-Ramirez, Long-term nose-only cigarette smoke exposure induces emphysema and mild skeletal muscle dysfunction in mice, Dis. Model. Mech. 5 (2012) 333 LP–341, https://doi.org/10.1242/dmm.008508.
[42] C. Asti, V. Ruggieri, S. Porzio, R. Chiusaroli, G. Melillo, G.F. Caselli, Lipopolysaccharide-induced lung injury in mice I. Concomitant evaluation of inflammatory cells and haemorrhagic lung damage, Pulm. Pharmacol. Ther. 13 (2000) 61–69, https://doi.org/10.1006/pupt.2000.0231.
[43] H. Falk, A. Smailagic, C. Brunmark, A. Miller-larsson, H. Lal, Pulmonary pharmacology & therapeutics altered lung function relates to inflammation in an acute LPS mouse model q, Pulm. Pharmacol. Ther. 25 (2012) 399–406, https://doi.org/10.1016/j.pupt.2012.08.001.
[44] T. Chen, Y. Mou, J. Tan, L. Wei, Y. Qiao, T. Wei, P. Xiang, S. Peng, Y. Zhang, Z. Huang, H. Ji, International immunopharmacology the protective effect of CDDO-Me on lipopolysaccharide-induced acute lung injury in mice, Int. Immunopharmacol. 25 (2015) 55–64, https://doi.org/10.1016/j.intimp.2015.01.011.
[45] D. Souza, X. Costa, G.R. Ju, A. Aparecida, L. Belotti, D.H. Zati, M.F. Cavalcante, M.M. Veras, S. Ribeiro, E.G. Kalla, N. Saldiva, M. Dolhnikoff, L. Fernando, Early and late pulmonary effects of nebulized LPS in mice : an acute lung injury model, PLoS One 12 (2017) e0185474.
[46] L. Swedin, S. Dahle, M. Starkhammar, S.K. Geore, L.O. Cardell, Intranasal Administration of poly (I : C) and LPS in BALB / c mice induces airway hyperresponsiveness and inflammation via different pathways, PLoS One 7 (2012) e32110, , https://doi.org/10.1371/journal.pone.0032110.
[47] X. Hu, Y. Tian, S. Qu, Y. Cao, S. Li, W. Zhang, Z. Zhang, Protective effect of TM6 on LPS- induced acute lung injury in mice, Sci. Rep. (2017) 1–10, https://doi.org/10.1038/s41598-017-00551-8.
[48] D.M. Brass, J.W. Hollingsworth, M. Cinque, Z. Li, E. Potts, E. Toloza, W.M. Foster, D.A. Schwartz, Chronic LPS inhalation causes emphysema-like changes in mouse lung that are associated with apoptosis, Am. J. Respir. Cell Mol. Biol. 39 (2008) 584–590, https://doi.org/10.1165/rcmb.2007-0448OC.
[49] J.H.J. Vernooy, M.A. Dentener, R.J. Van Suylen, W.A. Buurman, E.F.M. Wouters, Long-term intratracheal lipopolysaccharide exposure in mice results in chronic lung inflammation and persistent pathology, Am. J. Respir. Cell Mol. Biol. 26 (2002) 152–159.
[50] H.A. Baarsma, S. Bos, H. Meurs, K.H. Visser, M. Smit, A.M.W.J. Schols, R.C. Langen, H.A.M. Kerstjens, R. Gosens, Pharmacological inhibition of GSK-3 in a guinea pig model of LPS-induced pulmonary inflammation: I. Effects on lung remodeling and pathology, Respir. Res. 14 (2013) 113, https://doi.org/10.1186/1465-9921-14-113.
[51] S.H. Al-Sheraji, A. Ismail, M.Y. Manap, S. Mustafa, R.M. Yusof, F.A. Hassan, Prebiotics as functional foods: a review, J. Funct. Foods 5 (2013) 1542–1553, https://doi.org/10.1016/j.jff.2013.08.009.
[52] L.V. Hooper, D.R. Littman, A.J. Macpherson, Interactions between the microbiota and the immune system the gut microbiota interactions between the microbiota and the immune system, Science 336 (2012) 1268–1273, https://doi.org/10.1126/science.1223490.
[53] H.J. Wu, E. Wu, The role of gut microbiota in immune homeostasis and autoimmunity, Gut Microbes 3 (2012) 4–14, https://doi.org/10.4161/gmic.19320.
[54] K.F. Budden, S.L. Gellatly, D.L.A. Wood, M.A. Cooper, M. Morrison, P. Hugenholtz, P.M. Hansbro, Emerging pathogenic links between microbiota and the gut-lung axis, Nat. Rev. Microbiol. 15 (2017) 55–63, https://doi.org/10.1038/nrmicro.2016.142.
[55] K.A.T. Verheijden, L.E.M. Willemsen, S. Braber, T. Leusink-muis, D.J.M. Delsing, J. Garssen, A.D. Kraneveld, G. Folkerts, Dietary galacto-oligosaccharides prevent airway eosinophilia and hyperresponsiveness in a murine house dust mite-induced asthma model, Respir. Res. 16 (2015) 1–9, https://doi.org/10.1186/s12931-015-0171-0.
[56] K.A.T. Verheijden, L.E.M. Willemsen, S. Braber, T. Leusink-Muis, P.V. Jeurink, J. Garssen, A.D. Kraneveld, G. Folkerts, The development of allergic inflammation in a murine house dust mite asthma model is suppressed by synbiotic mixtures of non - digestible oligosaccharides and Bifidobacterium breve M - 16V, Eur. J. Nutr. 55 (2016) 1141–1151, https://doi.org/10.1007/s00394-015-0928-8.
[57] M.A. Schijf, D. Kruijsen, J. Bastiaans, F.E.J. Coenjaerts, J. Garssen, G.M. van Bleek, B. van't Land, Specific dietary oligosaccharides increase Th1 responses in a mouse respiratory syncytial virus infection model, J. Virol. 86 (2012) 11472–11482, https://doi.org/10.1128/JVI.06708-11.
[58] Z.A. Ktsoyan, M.S. Mkrtchyan, M.K. Zakharyan, A.A. Mnatsakanyan, K.A. Arakelova, Z.U. Gevorgyan, A.M. Sedrakyan, A.I. Hovhannisyan, A.A. Arakelyan, R.I. Aminov, Systemic concentrations of short chain fatty acids are elevated in salmonellosis and exacerbation of familial mediterranean fever, Front. Microbiol. 7 (2016) 1–10, https://doi.org/10.3389/fmicb.2016.00776.
[59] E. Ciarlo, T. Heinonen, J. Herderschee, C. Fenwick, M. Mombelli, D. Le Roy, T. Roger, Impact of the microbial derived short chain fatty acid propionate on host susceptibility to bacterial and fungal infections in vivo, Sci. Rep. 6 (2016) 1–15, https://doi.org/10.1038/srep37944.
[60] B.J. Marsland, G.S. Eva, E.S. Gollwitzer, The gut – lung Axis in respiratory disease, Ann. Am. Thorac. Soc. 12 (2015) 150–156, https://doi.org/10.1513/AnnalsATS.201503-133AW.
[61] M.A.R. Vinolo, H.G. Rodrigues, R.T. Nachbar, R. Curi, Regulation of inflammation by short chain fatty acids, Nutrients 3 (2011) 858–876, https://doi.org/10.3390/nu3100858.
[62] P. Akbari, S. Braber, A. Alizadeh, K.A.T. Verheijden, M.H.C. Schoterman, A.D. Kraneveld, J. Garssen, J. Fink-Gremmels, Galacto-oligosaccharides protect the intestinal barrier by maintaining the tight junction network and modulating the inflammatory responses after a challenge with the mycotoxin deoxynivalenol in human Caco-2 cell monolayers and B6C3F1 mice, J. Nutr. 145 (2015) 1604–1613, https://doi.org/10.3945/jn.114.209486.
[63] A. Alizadeh, P. Akbari, E. Difilippo, H.A. Schols, L.H. Ulfman, M.H.C. Schoterman, J. Garssen, J. Fink-Gremmels, S. Braber, The piglet as a model for studying dietary components in infant diets: effects of galacto-oligosaccharides on intestinal functions, Br. J. Nutr. 115 (2016) 605–618, https://doi.org/10.1017/S0007114515004997.
[64] S. Varasteh, S. Braber, J. Garssen, J. Fink-Gremmels, Galacto-oligosaccharides exert a protective effect against heat stress in a Caco-2 cell model, J. Funct. Foods 16 (2015) 265–277, https://doi.org/10.1016/j.jff.2015.04.045.
[65] E.P.A. Rutten, K. Lenaerts, W.A. Buurman, E.F.M. Wouters, Disturbed intestinal integrity in patients with COPD: effects of activities of daily living, Chest 145 (2014) 245–252, https://doi.org/10.1378/chest.13-0584.
[66] H. Fukui, Increased intestinal permeability and decreased barrier function: does it really influence the risk of inflammation? Inflamm. Intest. Dis. 1 (2016) 135–145, https://doi.org/10.1159/000447252.
[67] M.J. Gnoth, S. Rudloff, C. Kunz, R.K.H. Kinne, Investigations of the in vitro transport of human milk oligosaccharides by a Caco-2 monolayer using a novel high performance liquid chromatography-mass spectrometry technique, J. Biol. Chem. 276 (2001) 34363–34370, https://doi.org/10.1074/jbc.M104805200.
[68] T. Eiwegger, B. Stahl, P. Haidl, J. Schmitt, G. Boehm, E. Dehlink, R. Urbanek, Z. Szépfalusi, Prebiotic oligosaccharides: in vitro evidence for gastrointestinal

epithelial transfer and immunomodulatory properties, Pediatr. Allergy Immunol. 21 (2010) 1179–1188, https://doi.org/10.1111/j.1399-3038.2010.01062.x.
[69] K.C. Goehring, A.D. Kennedy, P.A. Prieto, R.H. Buck, Direct evidence for the presence of human milk oligosaccharides in the circulation of breastfed infants, PLoS One 9 (2014) e101692, , https://doi.org/10.1371/journal.pone.0101692.
[70] E. Difilippo, M. Bettonvil, R.A.M. Willems, S. Braber, J. Fink-Gremmels, P.V. Jeurink, M.H.C. Schoterman, H. Gruppen, H.A. Schols, Oligosaccharides in urine, blood, and feces of piglets fed milk replacer containing galacto-oligosaccharides, J. Agric. Food Chem. 63 (2015) 10862–10872, https://doi.org/10.1021/acs.jafc.5b04449.